Effect of Gamma Rays on the Chromosomes of the Somatic Cells in Picea Abies Karst.*)

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(Received for publication November 19, 1962)

Introduction

In plant breeding the application of ionizing radiations reprcsents an ever more current method of working. Irradiation can produce inheritable changes in the plant. Therefore tree breeders make use of this working method in order to obtain positive mutants.

Availing ourselves of these achievements our activities in the breeding of Norway Spruce *(Picea Abies* Karst.) are performed also in this direction i. e. obtaining of positive mutants by means of ionizing radiation. Because of the recent date of introduction of this method in breeding forest tree species, and considering the still rather modest results in this field, we set ourselves the task to observe the changes produced in the somatic and sexual cells of Norway Spruce by gamma radiation. We feel that these investigations will give us a better insight into the dose requirements of gamma radiation applied to Norway Spruce to induce mutations.

In this work are presented our first results as to the effect of gamma radiation on the somatic cells of Norway Spruce. They have not yet been completed but are most interesting and considered by us to be useful for publication.

Working Method

The Norway Spruce seeds marked S_{55} which we used for our investigations were collected in a natural population growing in the region of Delnice, Gorski Kotar (Northwestern part of Croatia). Seeds were irradiated with gamma rays of Co_{60} from a source of 1000 C. The radiation doses were 100, 500, 1000, 3000, 5000, 7500, and 10000 r. The irradiations were performed always under the same geometrical conditions. The humidity of the seeds-measured before the irradation — amounted to 7.35%. After the radiation the seed was stored in a refrigerator at a temperature of 4^0 C, and it was used for tests when required.

The preparation of the material for the cytological treatment was performed in the following way: the seeds were put for germination into a Petri dish and watered with ordinary water. After the emergence and when the roots achieved a length of 3–4 mm, they were cut and subjected first to the so-called pretreatment, i. e. put into a 0.3% water solution of colchicine. After three hours the roots were soaked for 24 hours into an aceticalcohol fixative, and thereafter passed through 70% alcohol and stored into a refrigerator. The material used at once was not put into the last mentioned fixative, but after the aceticalcohol we immediately proceeded to further treatment i. e. to the hydrolisis by means of 1 N HCl at 60^0 C during about one hour. After hydrolisis the slides were prepared by the carmine-acidsquash method.

The morphology of chromosomes was examined in the stage of the late prophase and metäphase respectively, and in addition to that also some changes in the anaphase were observed. The chromosomes were drawn by means of a drawing apparatus, and thereafter their lengths measured and given in mm. As the cells were taken from the whole of the root tip, and as we know that the cells of the outer and inner layers distinguish among themselves, it is understandable, that the chromosomes too will differ as to their length. Therefore it is not possible to compare the absolute lengths but only the relative ones which were obtained by dividing the absolute lengths of each particular chromosome by the sum (Σ), i. e. by the total sum of the absolute lengths of all chromosomes e. g. : $1 : \Sigma$, $2 : \Sigma$ etc. The obtained quotient was always lower than 1, i. e. 0.0.... For the purpose of an easy orientation and drawing the decimal point was moved for three places so that e. g. 0.068719 became 68.719; there were taken into consideration only two decimals, while the third served only as a correction of the preceding.

Many cells were photographed and some of these photographs are to be found in this work.

Results of Investigations

The measurement of lengths of chromosomes — arranged according to size with ordinal number from 1 to 24 — was performed in the manner that the longest was numbered 1, and the shortest 24. If there occurred more chromosomes they were given further numbers.

The number of cells with a normal number of chromosomes in which was measured the relative lengths of chromosomes was the following: controls nine cells, seven cells — 100 r, five cells — 500 r, five cells — 1000 r, four cells — 3000 r, three cells — 5000 r, and two cells — 10000 r. In *Table* 1 are given the numerical data on the relative chromosome lengths of these cells. On the ground of these data were constructed the graphs on the relative lengths of the chromosomes for individual dosages and the controls.

In roots from seeds irradiated with doses from 3000 r and more, a fragmentation of chromosomes in several cells was produced while for dosages of 100, 500, and 1000 r no cell with fragments could be found. For dosage of 3000 r only one cell was found exhibiting 'but one fragment. At 5000 r one cell had 25 chromosomes, the second one two fragments and the third one only one fragment. A very interesting cell was the one irradiated 7500 r which showed 25 chromosomes and five fragments *(Fig.* 14). For the dose of 10000 r two cells with fragments were found, the one with 25 chromosomes and one fragment and the other cell with four fragments *(Fig.* 15). In *Table* 2 are given the relative lengths of chromosomes and fragments of these cells.

Larger dosages i. e. from 3000 r and more have brought about also some other abnormalities in mitosis. For 3000 r

*) The investigations were supported by the Federal Nuclear Energy Commission and the Federal Research Work Fund.

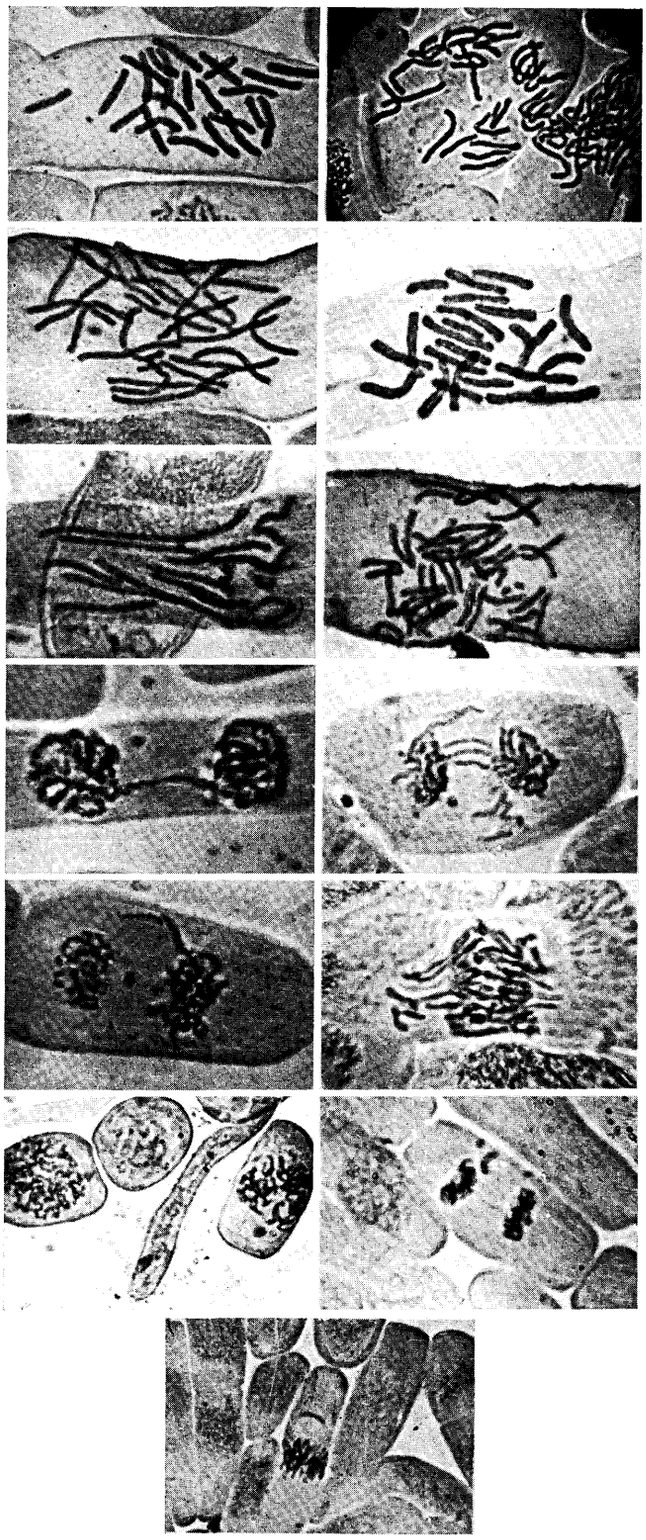

Fig. 1—13. — Microphotographs (from left to right): — *Fig. 1.* Cell of control with 24 chromosomes (× ca. 1000). — *Fig. 2.* Cell of 100 r with 24 chromosomes (× ca. 1000). — *Fig. 3.* Cell of 500 r with 24 chromosomes (× ca. 1000). — *Fig. 4.* Cell of 3000 r with 24 chromosomes (× ca. 1000). — *Fig. 5.* Cell of 3000 r with reduced numbers of extended chromosomes (× ca. 1000). — *Fig. 6.* Cell of 3000 r with double number of chromosomes in pairs (× ca. 1000). — *Figs. 7 and 8.* Cells of 7500 r with bridge (× ca. 1000). — *Fig. 9.* Cell of 7500 r with two chromosomes outside the spindle (× ca. 1000). — *Fig. 10.* Cell of 7500 r with abnormality in anaphase (× ca. 1000). — *Fig. 11.* Cell of 10000 r with chromosome erosion (× ca. 200). — *Fig. 12.* Cell of 10000 r with bridge and one chromosome outside the spindle (× ca. 200). — *Fig. 13.* Cell of 10000 r with one chromosome outside the spindle (× ca. 200).

Fig. 14. — Cell of 7500 r with 25 chromosomes and 5 fragments.

Fig. 15. — Cell of 10000 r with 24 chromosomes and 4 fragments.

one cell shows for instance a reduced number of somewhat extended chromosomes *(Fig. 5)*, while another one also for 3000 r — shows a double number of chromosomes *(Fig. 6)*. Abnormalities appeared also in the stage of anaphase as well as in the anaphase-telophase *(Fig. 9, 10)*. In this way we obtained at doses of 5000, 7500, and 10000 r in a larger number of cells well-pronounced bridges *(Fig. 7, 8, 12)*.

The radiation also influenced the time necessary for the seed to germinate. As visible from *Table 3*, the seed irradiated in dosages of 100 to 3000 r requires in general the same time for germination as the controls, while at larger dosages — from 5000 r to 10000 r — this interval roughly more than doubled.

Discussion

If on the graph representing the relative chromosome lengths we observe the curves of all radiation doses, and if we compare them with the controls, we can notice that the irradiation influenced the changes of chromosome lengths always in the same manner, i. e. in all doses larger changes were produced on the extreme chromosomes — on the smallest and largest ones — than on the medium-sized ones. This is visible on the *graph (Fig. 17)* by the fact that on these places the curves are more distant from one another than are in the very middle of the graph,

Table 1.

Serial No. of Chromosomes	Relative lenght of chromosomes						
	Control	100 r	500 r	1000 r	3000 r	5000 r	10 000 r
1	0 060836	0.062788	0.060059	0,057156	0 052993	0.065683	0.058750
2	0 055963	0.057034	0.055221	0.053222	0 051729	0.061253	0.057132
3	0.053881	0.053326	0.052052	0.051189	0.050734	0.057822	0.054216
4	0 052164	0.051892	0.050073	0.049649	0.048919	0.055105	0.053322
5	0,050341	0.051137	0.049080	0.047837	0.048699	0 050726	0 052275
6	0.048484	0.049993	0.048080	0.046754	0.047143	0.049838	0.051413
7	0.047666	0.049079	0 047116	0.046152	0 046017	0 048069	0 048868
8	0 047127	0.047663	0.045985	0.045266	0 045004	0 046991	0.046942
9	0 046055	0.046758	0,045312	0.044070	0.044199	0.045333	0 045895
10	0.045007	0.046068	0.044326	0.043396	0.043825	0.043905	0 045226
11	0.043903	0 044505	0 043475	0.042886	0.043322	0.043437	0 044952
12	0 042744	0.042868	0.042369	0.042655	0.042765	0.042074	0.043800
13	0.041788	0.041511	0.041812	0.041682	0 041504	0.041303	0 042818
14	0.040511	0.040594	0.040511	0.040859	0 041101	0 040483	0.040739
15	0.039193	0 039201	0 039772	0.039748	0.040407	0.038937	0 039007
16	0 038034	0.038389	0.038920	0.039239	0.039959	0 037273	0.036196
17	0 036413	0.037494	0 037034	0 038758	0.039219	0 036455	0 035939
18	0.035159	0 035178	0 035989	0.037510	0.038276	0.035712	0.035286
19	0 033720	0 033436	0 034967	0.036365	0 037333	0 034376	0 032682
20	0 033078	0 029747	0.032925	0.035524	0 035417	0 029860	0.032046
21	0 031097	0.028147	0.031469	0 034207	0.033572	0 027627	0 029517
22	0.028889	0 026875	0.029450	0 032781	0.031414	0 025844	0.027648
23	0 025531	0.025058	0 028855	0.029077	0.029322	0 024091	0.023595
24	0.022414	0.021258	0.025145	0.024019	0.027125	0.017788	0.021741

Table 2.

Serial number of chromosome	Relative lenght of chromosomes and fragments						
	3000 r	5000 r			7500 r	10000 r	
	1 cell	1 cell	1 cell	1 cell	1 cell	1 cell	1 cell
1	0 063479	0.061239	0.079273	0.056812	0 061428	0 068543	0.069347
2	0.055769	0.058918	0 052146	0.056812	0.057951	0 065965	0 068922
3	0 051401	0.052134	0.049513	0.054718	0.056328	0.056940	0.062753
4	0.049345	0.051955	0 048986	0.051926	0.055865	0.050279	0 046586
5	0.047803	0.051062	0.046352	0 050530	0.051692	0 047916	0.045735
6	0 047189	0.049277	0 044509	0.050251	0.050997	0.047271	0.045097
7	0.046518	0.045885	0.044245	0 045924	0.049374	0 046841	0.044671
8	0 045747	0 045528	0.042402	0.044668	0.048679	0 046197	0 044033
9	0.044719	0.044635	0 040822	0 043969	0.041956	0.045982	0.043608
10	0 044205	0 043207	0 040558	0.042574	0 041956	0.045122	0 042969
11	0.043691	0.042671	0 040558	0.041318	0 041029	0 040825	0 042119
12	0.043691	0.041778	0.039242	0 040619	0.039870	0 040325	0.039566
13	0.042663	0.041421	0.038978	0.039782	0 039407	0 038032	0.037226
14	0.041635	0.040707	0.038451	0.039224	0.038248	0.037817	0 035949
15	0.040607	0.039636	0.037398	0 038945	0.037320	0.035453	0.035312
16	0.039065	0.037672	0.037135	0 037269	0.037089	0 035453	0 035099
17	0.037779	0 033029	0.037135	0.036153	0.036393	0 033949	0 034035
18	0 035209	0 032137	0 034501	0.034059	0 030829	0 031156	0.032972
19	0.033667	0.032137	0 034238	0.033780	0 027816	0 028148	0 029143
20	0.030326	0.028566	0 031867	0 033640	0.025962	0.026644	0 028717
21	0 029041	0 028031	0 031077	0.030709	0 025267	0 026214	0 028079
22	0 028784	0 026602	0 030287	0.030011	0.025267	0 025569	0 025739
23	0 025186	0 026245	0.028970	0 028197	0 020399	0 024495	0.024037
24	0.021074	0.026245	0 027917	0.026382	0.017385	0.022991	0.023825
25			0,023439		0 017385	0 020842	
Fragments	0.011308	0.011962		0.011725	0 007649	0.010529	0 012976
		0.007320			0 006259		0 011274
					0 004636		0 006807
					0 002782		0.003404
					0.002782		

i. e. in the medium-sized chromosomes with serial numbers from 12 to 14. If dividing all chromosomes into three groups viz. 1—8, 9—16, and 17—24, we see that the largest changes occurred in the first and last groups. Consequently we may suppose that the medium-sized chromosomes are more stable than the largest and the smallest ones, which accordingly are more easily subject to changes of length.

From the same *graph (Fig. 17)* also the following can be observed : the curves on the graph which up to the half-way are running above the line of controls, deviate — past the half-way (i. e. after 12 chromosomes, actually between the chromosomes 12 and 14) and after intersecting the curve of the controls — into another direction running then below the line of the controls. The same is valid for those curves which go first below the line of the controls, and then take another direction and run above it. In other words, when the irradiation induced the largest chromosomes in the way to increase, the smallest were induced to shorten, and conversely, when the largest chromosomes became shorter, the shortest ones became larger. It would seem that there is a certain regularity here. This is corroborated also by the following fact. Through a summation of all relative values — for each dose separately — we obtained almost the same values. The differences occur

Fig. 16. — Cell of 5000 r with chromosome erosion.

Table 3.

Dose r	Germination time (days)
10000	13
7500	10
5000	12
3000	5
1000	6
500	5
100	5
Control	5

only in the fourth decimal *(Table 4)*. This indicates that the chromosomes matter remained quantitatively the same, because the more the large chromosomes became shortened owing to the irradiation, the more the shorter ones became longer, and conversely.

Hence we see from this *graph (Fig. 17)* that the abnormalities produced on various chromosomes are different. Unequal changes which occurred here show us yet another interesting phenomenon which happened just because of unequal changes in the lengths of chromosomes. It is visible on the individual curves on the graph in the form of larger or smaller breaks which exist among the individual chromosomes. In the controls there occur the smallest number of breaks, and they are a reflection of the sizes of chromosomes viz. of the karyotype of species. Contrariwise, in the cells from irradiated seeds the breaks on the curves are more numerous, i. e. these curves do not run always parallel with the curve of controls but make in places smaller or larger deviations in the form of smaller or greater breaks, which indicates that the irradiation produced changes in the chromosome lengths, which is reflected in the curves in the form of breaks.

In the preceding chapter we have pointed out that at

Table 4.

Dose r	Sum of the relative lenght of chromosomes
100	0.999999
500	0.999997
1000	1.000001
3000	0.999998
5000	0.999985
10000	1.000005
Control	0.999958

doses over 1000 r there came to a fragmentation of chromosomes as well as to other abnormalities in mitosis. SUSZKA, OHBA and SIMAK (17) have reported for the Scots Pine that the number of abnormalities in mitosis augments with an increase of X-ray doses. To the same conclusion came SAX and BRUMFIELD (15) for the *Vicia faba* species when its seeds and germinated embryos were irradiated by X-rays. EHRENBERG, GUSTAFSSON, LEVAN and VON WETTSTEIN (3), applying the so-called *Allium*-test, came to the conclusion that with the increase of radiation doses there increased also the number of abnormalities in division of cells.

Our results evidence the same phenomenon. At smaller doses (100, 500 and 1000 r) there occurred in all cases only changes in the relative lengths of the chromosomes in comparison to the controls, while at larger doses (3000, 5000, 7500, and 10000 r) there came to a fragmentation of the chromosomes as well as to other abnormalities in mitosis.

Obtained were also the cells with a double number of chromosomes. We achieved such a characteristic example at 3000 r-dose, where the chromosomes were arranged in a sort of pairs *(Fig. 6)*. A similar case is described by MERGEN and LESTER (10) for nine species of *Abies*, and by MERGEN (9) for four species of Pines, when the socalled C-pairs were induced through the action of colchicine.

Because of irradiation there appears according to LEVAN (8) also a kind of chromosomal aberration, the so-called chromosome erosion. This phenomenon was established also by EHRENBERG, GUSTAFSSON, LEVAN and VON WETTSTEIN (3). In our material — in doses from 5000 r to 10000 r — were found cells whose chromosomes exhibit a structure which would correspond to that of chromosome erosion *(Fig. 11, 16)*.

Summary

Seeds of Norway Spruce (*Picea Abies* KARST.) were irradiated by gamma rays of Co_{60}. The radiation doses were 100, 500, 1000, 3000, 5000, 7500 and 10000 r. The observation of mitosis was carried out in cells of root tips of irradiated and nonirradiated seeds.

It was established that in cells produced from the irradiated seeds there occurred changes of relative lengths of chromosomes in comparison to the controls. In all these doses the irradiation produced changes of chromosome lengths so that larger changes in the chromosome length occurred always in larger and smaller chromosomes, while in the medium-sized ones there occurred smaller changes. It is characteristic that the over-all length of chromosomes of the individual cells — of various dosages — did not change in comparison with the controls.

In doses from 100 to 1000 r there came to the changes of lengths only of individual chromosomes in comparison with the controls. In larger doses, i. e. from 3000 to 10000 r there occurred a fragmentation of chromosomes as well

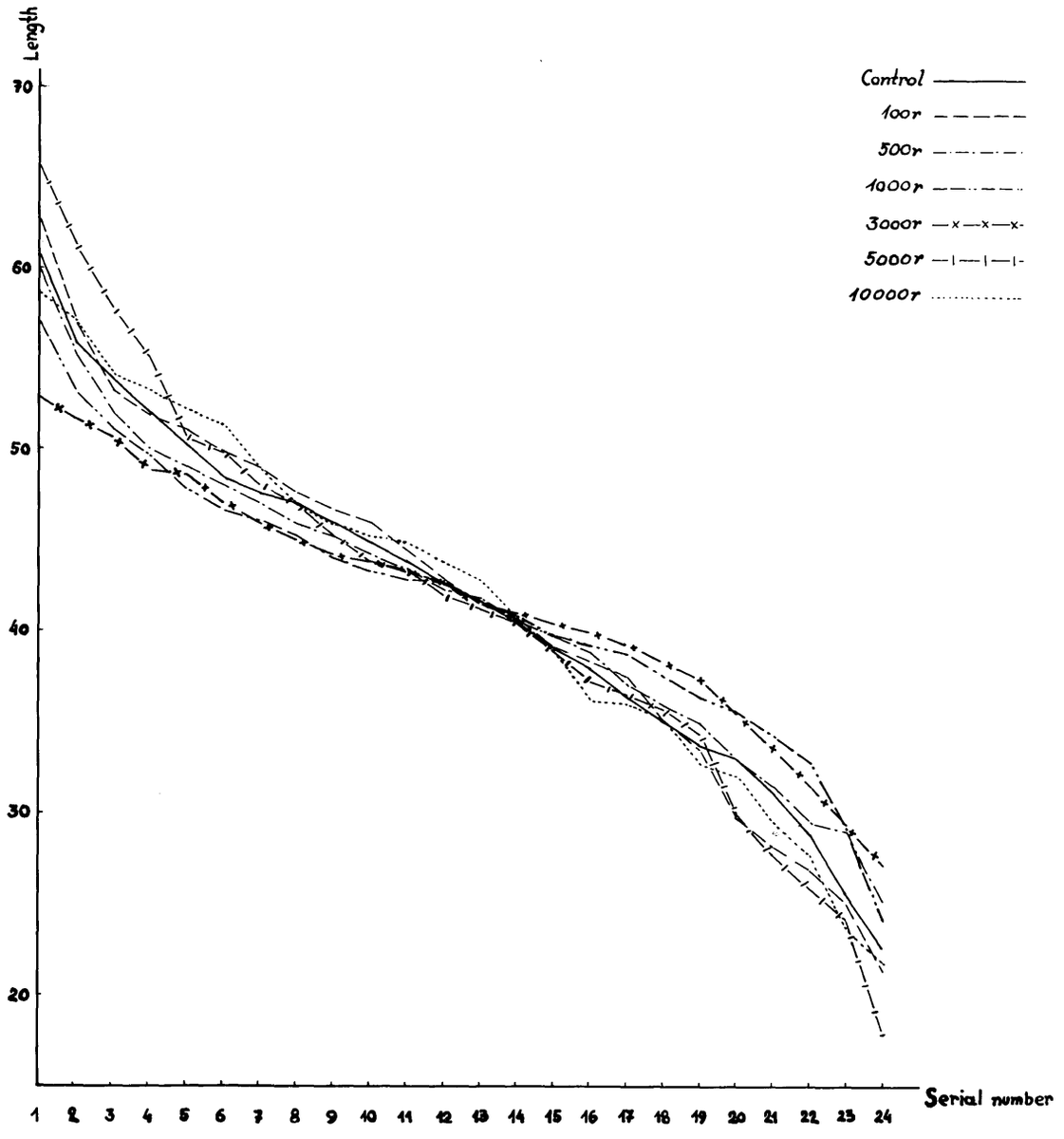

Fig. 17. — Relative lengths of chromosomes.

as other abnormalities in mitosis. Obtained were cells with a smaller number of chromosomes, as well as cells with a double number of chromosomes arranged in pairs.

In doses of 5000, 7500 and 10000 r the larger number of cells in the stage of anaphase showed bridges, and there occurred also other abnormalities in this stage as well as in the anaphase-telophase.

Résumé

Titre de l'article: *Effet des rayons gamma sur les chromosomes des cellules somatiques de Picea Abies Karst.*

Les graines d'Epicéa commun (*Picea Abies* Karst.) ont été irradiées par les rayons gamma de Co_{60}. Les doses d'irradiation sont les suivantes: 100, 500, 1000, 3000, 5000, 7500 et 10000 r. La mitose a été étudiée dans les cellules prises de la pointe de racines des graines irradiées et non irradiées.

On a constaté que dans les cellules tirées des graines irradiées des changements de longueurs relatives de chromosomes se sont produits par rapport aux cellules-témoins (*Tab. 1*). Des changements de longueurs de chromosomes se sont produits dans toutes les doses d'irradiation de telle façon que les changements plus grands se sont manifestés chez les chromosomes grands et petits que chez les chromosomes moyens. Il est à noter que la longueur d'ensemble des chromosomes de chaque cellule — de différentes doses — n'a pas changé en comparaison avec les cellules-témoins (*Tab. 4*).

Dans des doses de 100 à 1000 r ils ne se sont produits que des changements de longueurs chez des chromosomes particuliers par rapport aux cellules-témoins. Dans des doses plus grandes, c'est-à-dire de 3000 à 10000 r une fragmentation des chromosomes ainsi que d'autres anomalies à la mitose se sont produites. On est arrivé d'une part à des cellules à nombre reduit de chromosomes et de l'autre à des cellules au nombre doublé des chromosomes classés en paires.

Dans des doses de 5000, 7500 et 10000 r un grand nombre de cellules a donné des fuseaux à l'anaphase. D'autres anomalies y se sont manifestées ainsi qu'à la transition de l'anaphase à la telophase.

Zusammenfassung

Titel der Arbeit: *Über die Auswirkung von Gamma-Strahlen auf die Chromosomen somatischer Zellen bei der Fichte.*

Samen der gemeinen Fichte *(Picea Abies* KARST.) wurden mit Gamma-Strahlen von Co_{60} bestrahlt. Als Strahlungsdosen wurden verwandt: 100, 500, 1000, 3000, 5000, 7500 und 10000 r. Die Mitose-Beobachtungen sind an Wurzelspitzen-Zellen bestrahlter und unbestrahlter Samen durchgeführt worden.

Es wurde festgestellt, daß es bei den aus bestrahlten Samen stammenden Zellen zu Veränderungen der relativen Chromosomen-Längen gegenüber den Kontrollen gekommen war *(Tab. 1)*. Bei allen Bestrahlungsdosen wurden Chromosomen-Längenveränderungen in der Weise bewirkt, daß die stärkeren Abweichungen immer bei den großen und kleinen Chromosomen vorkamen, während bei den mittelgroßen Chromosomen nur kleinere Veränderungen auftraten. Dagegen hatte sich die Gesamtlänge der Chromosomen der einzelnen Zellen — bei verschiedenen Dosen —, verglichen mit den Kontrollen, nicht verändert *(Tab. 4)*.

Bei Anwendung der Dosen 100 r bis 1000 r ist es nur bei einzelnen Chromosomen zu Längenabänderungen gekommen. Bei den größeren Dosen, d. h. von 3000 r bis 10000 r, kam es zu Chromosomenfragmentierungen sowie zu anderen Anomalien der Mitose. Es wurden sowohl Zellen mit kleinerer Chromosomenzahl als auch Zellen mit doppelter Chromosomenzahl, die paarweise angeordnet waren, erhalten.

Bei den Dosen 5000 r, 7500 r und 10000 r zeigte eine größere Anzahl Zellen im Stadium der Anaphase Brücken-Bildungen. Ferner kamen noch weitere Anomalien in diesem Stadium und auch im Übergangsstadium Anaphase-Telophase vor.

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The Degree of Natural Selfing in Slash Pine as Estimated from Albino Frequencies[1])

By A. E. SQUILLACE and J. F. KRAUS[2])

(Received for publication October 29, 1962)

The extent of selfing in forest trees is of interest to tree improvement workers because inbreeding often results in depression of vigor and other economically important traits. Although the overall importance of selfing has not yet been evaluated, it is of special interest where clonal orchards are used as the means of mass producing seed of a superior strain. In such orchards the potential for selfing is magnified because selfs can not only be produced from selfing of a ramet but also from mating of ramets of the same clone. Reliable knowledge of the extent of selfing that occurs under natural conditions will help to show the extent of selfing that may occur in seed orchards.

Although most pines are generally considered to be largely crosspollinated, there have been few experiments designed to estimate the relative amount of selfing vs. crossing that occurs under natural conditions. The assumption of the predominance of crossing is largely based on studies of natural barriers to selfing, such as dichogamy, and evidences of reduced seed or seedling yield from isolated trees and from artificial selfing. Such evidence does not, of course, provide a reliable estimate of the actual degree of natural selfing.

Many studies have shown that most coniferous species can be successfully selfed under artificial conditions. Seed and seedling yields from selfing are usually low. However, there is considerable variation in self-fertility both between species and among individual trees within species. Serbian spruce *(Picea omorika* [PANČIČ] PURKYNE), for example, has been shown to be highly self-fertile as tested under artificial pollination (LANGNER, 1959). Most western white pines *(Pinus monticola* DOUGL.) show a depressed seed yield from artificial selfing, but some individuals will yield fully as many seed and seedlings under selfing as under outcrossing (BINGHAM and SQUILLACE, 1955).

[1]) The authors gratefully acknowledge the assistance of the following organizations in collecting the seed used in this study: Buckeye Cellulose Corp., Foley, Florida; Continental Can Co., Inc., Savannah, Georgia; Florida Forest Service; Georgia Forestry Commission; International Paper Co., Bainbridge, Georgia; School of Forestry, University of Florida; South Carolina Commission of Forestry; West Virginia Pulp and Paper Corp., Georgetown, South Carolina. They are likewise grateful to the following for reviewing the manuscript: BURTON V. BARNES, R. T. BINGHAM, D. P. FOWLER, RAY E. GODDARD, E. BAYNE SNYDER, and A. T. WALLACE.

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